Standard Operating Procedure for Zooplankton Analysis

LG403

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Standard Operating Procedure for Zooplankton Analysis

1.0 SCOPE AND APPLICATION

1.1 This method, as developed from Gannon (1971), Stemberger (1979) and Evans *et al.* (1982), is used to identify and enumerate the zooplankton populations from the Great Lakes.

2.0 SUMMARY OF METHOD

2.1 The method involves microscopic examination of preserved zooplankton samples collected with a conical net towed vertically through a water column. Microcrustacea are examined in four stratified aliquots under a stereoscopic microscope. Rotifera are examined in two equal volume sub-samples under a compound microscope.

3.0 SAMPLE COLLECTION AND PRESERVATION

3.1 See U.S. EPA GLNPO Standard Operation Procedure (SOP) for Zooplankton Sample Collection and Preservation.

4.0 APPARATUS

4.1 Most supplies can be acquired from biological supply companies (such as Wildlife Supply Company). The supplies needed are as follows:

Dissecting microscope with 10x to 50x magnification
Compound microscope with 100x to 600x magnification
1-mL Calibrated Hensen-Stempel pipette
100-, 250- and 500-mL graduated cylinders
Folsom plankton splitter
Ward counting wheel or other suitable counting chamber
Sedgwick-Rafter counting cell
Cover glass for Sedgwick-Rafter counting cell
Microscope slides, 1 x 3 inch
Cover slips
Tubes for concentrating plankton samples (see below)
Small sieves with 63 and 500 µm mesh
63 µm Nitex mesh
Heavy duty rubber bulb
Microprobe
Micro-forceps
100- to 500-mL glass jars with split fractions written on labels (2-2048)

4.2 The plankton concentrating tube is constructed by covering one end of a wide glass tube (such as a chromatography tube) with 64 µm mesh. The mesh is secured with O-rings and a heavy-duty bulb is attached to the other end to provide suction.

5.0 REAGENTS

5.1 Reagents can be ordered through chemical supply companies. CMC has been acquired through Master's Company, Inc.

5.2 The reagents needed are as follows:

Formalin (37% formaldehyde solution)
Ethanol
5% Sodium hypochlorite solution (Clorox bleach)
CMC-9, CMC-10, Hoyer's or other suitable mounting medium for mounting and clearing slide-archived specimens
Rose Bengal stain dissolved in ethanol
Dilute solution of laboratory detergent

6.0 ANALYTICAL PROCEDURE – MICROCRUSTACEAN SAMPLE ANALYSIS

6.1 Microcrustacean Stratified Splitting

6.1.1 When zooplankton samples are returned to the lab, approximately 1 to 3 mL of Rose Bengal stain solution may be added to each sample to aid in finding the smaller organisms. Samples should be processed one at a time. Under the hood, rinse the sample from the sample bottle through a 63-µm mesh sieve with DI water to remove the formalin.

6.1.2 Be sure to rinse the sample bottle thoroughly with RO/DI/distilled water into the 63-µm mesh sieve to remove any residual organisms adhering to walls of the bottle. All containers from which zooplankton are transferred are to be rinsed thoroughly, including the Folsom splitter, glass jars, and counting chambers. Wash the sample into a glass jar. Adding a small amount of dilute laboratory soap to each sample at this time aids in preventing organisms from sticking to the sides of the containers and from floating at the surface of the sample.

6.1.3 If the sample contains large clumps of *Cercopagis*, then:

6.1.3.1 Carefully remove the clumps with forceps and place them on a very large mesh (approximately 500 µm).

6.1.3.2 Gently rinse the clumps with a stream of DI water, while pulling at the clumps with forceps to free trapped organisms.

6.1.3.3 Return "freed" organisms and rinse water to the sample and split as usual.

6.1.3.4 Reserve the clumps of *Cercopagis* in a separate jar and count all of the organisms, as there is no splitting method to deal with such clumps. Also note any *Bythotrephes* identified during this counting process.

6.1.4 Stir the sample gently to break up algal clumps and then pour the entire sample into the Folsom plankton splitter. Stir the sample again to distribute animals uniformly and split the sample by immediately rotating the splitter before the organisms can settle. Rinse the inside of the splitter well to remove organisms that may stick to the sides. Rinse one

sub-sample from the splitter receiving trays and save it in a labeled jar indicating the fraction of total original volume it contains (1/2).

6.1.5 The second sub-sample from the split is placed in the Folsom plankton splitter and divided again. One sub-sample is saved in a labeled jar indicating the fraction of the total original volume it contains (1/4).

6.1.6 Repeat Steps 6.1.3 and 6.1.4 as many times as necessary until the last 2 sub-samples contain at least 200 and no more than 400 microcrustaceans each (not including nauplii). These 2 sub-samples represent equal fractions of the original sample. One sub-sample is saved in a jar with the appropriately labeled split, and the other sub-sample is saved in a jar labeled "B".

6.2 Microcrustacean Enumeration

6.2.1 Four sub-samples are to be examined and enumerated. The sub-sample is concentrated by using the small sieve or the condensing tube and placed in a circular (or other suitable) counting chamber. All microcrustaceans are identified and enumerated under a dissecting microscope. The four sub-samples are counted using the criteria listed below in 6.2.1.1, 6.2.1.2, and 6.2.1.3. Refer to Figure 1 for a diagram of the splitting process.

6.2.1.1 The final two sub-samples which contain 200 - 400 organisms (see 6.1.6) are to be counted first. These are referred to as the A and B Counts. All microcrustaceans (except nauplii) are examined and enumerated. Measurements on selected individuals should be made at this time (Section 8.1). If the sub-samples contain a large amount of algae, it may be necessary to pick out the organisms and transfer them to a clean counting chamber prior to identification.

6.2.1.2 A third sample equal in fraction to the sum of the first two (A + B) samples is examined for subdominant taxa (taxa encountered less than 40 times in A and B counts combined). This is the C Count.

6.2.1.3 A fourth sub-sample equal in fraction to the sum of the first three (A, B, and C) counts is examined for large and rare taxa. This is the D count. Large and rare taxa include (but are not limited to) *Limnocalanus macrurus*, *Senecella calanoides*, *Epischura lacustris*, *Holopedium gibberum*, *Diaphanosoma birgei*, *Leptodora kindti* and *Polyphemus pediculus*. If a taxon defined as "large" has a sum of more than 40 individuals in counts A, B, and C, it is not necessary to enumerate them in D count. Note that *Mysis*, *Bythotrephes cederstroemi* and *Cercopagis pengoi* are enumerated separately.

6.2.1.4 The entire sample must be examined for *Mysis*, *Bythotrephes* and 'loose' *Cercopagis*. They can be removed from the sample prior to splitting if they are numerous. Otherwise they should be enumerated from A, B, C and D splits as they are examined, and their numbers noted. After these splits have been counted, pour the uncounted portion through a 500-µm mesh sieve. Examine the organisms trapped in the sieve for *Bythotrephes*. Enumerate them, add to this count any *Bythotrephes* encountered in previous counts, and record this on the bench sheet. This is the number of *Bythotrephes* in the entire count.

6.2.1.5 If *Cercopagis* or *Mysis*, are present, then the entire sample must be examined for loose animals. Enumerate all "loose" *Cercopagis* in the same manner as *Bythotrephes* is enumerated, and add this number to the *Cercopagis* counted in clumps. Note this sum on the bench sheet.

6.2.2 General Analysis Guidelines

6.2.2.1 Those organisms requiring higher magnification for identification are mounted on slides and examined at 100 - 1000x magnification under a compound microscope.

6.2.2.2 While counting Microcrustacea, make sure that all organisms are settled to the bottom. It is possible to sink floating Microcrustacea by gently pressing them down using the microprobe or by adding a drop of dilute laboratory detergent.

6.2.2.3 It is necessary to identify and record the sex of all mature Copepods encountered. This information is not presented in the final report, but is important for future reference.

6.2.2.4 When triplicate samples are collected in the field, all samples from that station should be analyzed by the same analyst. Shallow and deep tows from each station should also be analyzed by the same analyst.

6.2.2.5 If a sample cannot be completely counted and archived within 2 days, the sample should be kept in the refrigerator and a few drops of formalin can be added to the jars to prevent organisms from clumping. Sample analysis should not extent beyond four days.

6.2.2.6 In order to check for consistency of identification and enumeration, analysts can compare their microcrustacean and rotifer results with historical data. On some occasions, analysts may choose to re-examine archived samples in order to confirm identifications or to clarify taxonomic problems.

6.2.2.7 Occasionally, organisms are encountered which do not already appear on the species list. After the taxonomic status of such an organism is determined, the organism should be placed in a labeled vial and preserved with ethanol. The label in the vial should include genus/species name, date preserved, analyst initials, station number, and sample number. This will serve as a voucher specimen. The voucher specimen should be sent out for external confirmation, then a report made to the WAM including the distinguishing characteristics used to identify the new organism, and suggestions as to why it has not been encountered in the past (e.g., it is primarily benthic or littoral). Only AFTER written notification of acceptance of the new organism by the WAM should that species be added to the species list.

6.2.2.8 It is important that the voucher specimens are checked periodically so lost or damaged animals can be replaced. At least one male and one female (preferably 3 - 5) representative specimen should be available at all times for examination.

6.3 Taxonomic References

6.3.1 Adult calanoids are identified according to Balcer *et al.* (1984). Adult cyclopoids and Harpacticoids are identified according to Hudson *et al.* (1998). Immature calanoids and cyclopoids are identified to the lowest taxonomic level possible, usually suborder or genus. Nauplii are counted with rotifers. Malacostracans (i.e., *Mysis relicta*) are identified according to Balcer *et al.* (1984). Because malacostracans are predominantly benthic animals, they are enumerated and measured for historical purposes but not included in the final report. The following cladocerans are identified according to Balcer *et al.* (1984): *Leptodora kindti*, *Polyphemus pediculus*, *Holopedium gibberum*, and *Diaphanosoma birgei*. Brooks (1959) and Evans (1985) are used for all Daphnidae. The remaining cladocerans (Chydoridae, Bosminidae, and Macrothricidae) are classified according to Edmundson (1959). Members of Cercopagidae (i.e., *Bythotrephes cedarstroemii*, *and Cercopagis pengoi*) are identified according to Rivier (1998).

7.0 ANALYTICAL PROCEDURE – ROTIFER SAMPLE ANALYSIS

7.1 Rotifer and Nauplii Sub-sampling

7.1.1 Rotifers and nauplii are only counted from the tow taken with the 63 mm mesh net. Tows taken with the larger mesh (153 mm) will not capture sufficient numbers of the smaller rotifers.

7.1.2 Selection of the split level from which a sub-sample for rotifer enumeration is taken is based on estimates from previous samples within the data set, or from estimates made during microcrustacean enumeration (rotifers are visible in the dissecting microscope).

7.1.3 Two separate 1-mL sub-samples are taken from the appropriate split, and rotifers and nauplii are counted and identified separately from these two sub-samples. These are referred to as "A" and "B" counts. In cases where abundances are particularly low, more than one 1-mL sub-sample might be used for each count (see 7.1.7).

7.1.4 The sample should be mixed thoroughly, and a 1-mL sub-sample withdrawn with a Hensen-Stempel pipette (or other precalibrated large-bore pipette).

7.1.5 The 1-mL sub-sample should contain between 200 and 400 rotifers and crustacean nauplii.

7.1.6 If the sub-sample contains less than 200 organisms, a different sub-sample is taken from a jar with a larger fraction of the original sample volume. If the sub-sample contains more than 400 organisms, another sub-sample from a jar with a smaller fraction is used.

7.1.7 It is also permissible to use a second 1-mL aliquot if the original aliquot has less than 200 organisms. This second aliquot is counted in the same manner as the first and the results are combined to make A and/or B Count.

7.1.8 In cases of extremely low rotifer densities, the sample may be concentrated prior to taking sub-samples with the pipette. The maximum number of 1-mL aliquots counted at the lowest possible split level is 3 per count (i.e., a total of 6 mL), even if the sum does not reach 200 organisms.

7.2 Sedgwick-Rafter Cell Preparation and Rotifer Enumeration

7.2.1 The sub-sample is placed in a Sedgwick-Rafter cell and covered with a glass cover slip.

7.2.2 All rotifers, microcrustacean nauplii, and *Dreissena* veligers and post-veligers are identified and enumerated under a compound microscope at 100x magnification. Measurements on selected individuals should be made at this time (Section 8.2).

7.2.3 Veligers are enumerated for historical record but not included in the final report because of the variability in the reproductive cycle of *Dreissena*.

7.2.4 After the first rotifer count is completed, a second "duplicate" count, equal in volume to the first, is enumerated.

7.2.5 After the counts are completed, volume of the split used, including the volume of the aliquots, is measured, and this information is recorded.

7.3 Taxonomic References

7.3.1 Rotifers are identified to genus and to species where possible according to Edmonson (1959) and Stemberger (1976). Some rotifers may be indistinguishable by their gross morphology because of their contracted state; therefore, identification of these organisms is determined by examination of their chitinous mouthparts after using sodium hypochlorite bleach as a clearing agent (Stemberger 1979). This is a time-consuming process that destroys the rotifer and does not often produce clear results. Therefore, in an effort to use lab time efficiently, the bleaching process is most commonly used only as a training technique or in the instance of fairly common organisms with questionable identification.

7.4 Archiving Microcrustacean And Rotifer Samples

7.4.1 All crustacean and rotifer sub-samples are combined into a single jar. Depending on the amount of algal material suspended in the water column, the organisms are allowed to settle (usually overnight) and the surface water is siphoned off using a condenser tube or the sample is concentrated using the 63 µm mesh sieve.

7.4.2 The remaining combined sample is transferred to a 125-mL glass "Qorpak" bottle.

7.4.3 Fill the sample bottle close to the top with distilled water and add approximately 5 mL of formalin solution to the sample.

7.4.4 Label the bottle and the storage box with lake, station, lab number, and sample number. All archiving information should be computerized using a word processing program.

8.0 CALCULATION OF MICROCRUSTACEAN AND ROTIFER BIOMASS

8.1 Microcrustacean Biomass

8.1.1 Biomass (dry weight) of microcrustaceans is calculated from formulas relating some linear measurement (usually body length) to body weight. A compilation of the formula

references and constants can be found in Appendix 1. Formulas are derived from a number of sources, but are of the general form:

$$\ln w = \ln a + b \overline{\ln L}$$

where:

$\ln w$	=	natural logarithm of the dry weight estimate (µg)
$\ln a$ and b	=	species specific constants (listed in appendix)
$\overline{\ln L}$	=	the geometric mean length of measured individuals. This is calculated as the mean of the ln-transformed length measurements (L in mm)

8.1.2 Weights of *Mysis relicta* and *Bythotrephes cederstromi* are determined using the following relationships, developed by Shea and Makarewicz (1989) and Makarewicz and Jones (1990), respectively:

8.1.2.1 *Bythotrephes cederstromi*:

$$\ln w\ (\mu g) = 2.83 + 2.09\ \overline{\ln L}\ (mm)$$

8.1.2.2 *Mysis relicta*:

$$\ln w\ (mg) = -6.17093 + 2.86\ \overline{\ln L}\ (mm)$$

8.1.3 Weight of *Cercopagis pengoi* is determined using the following relationship developed by Ojaveer *et al*. (2001):

$$\ln w\ (mg) = -6.42 + 2.98\ \overline{\ln L}\ (mm)$$

8.1.4 Zooplankton may be measured by use of a calibrated eyepiece micrometer during the identification and enumeration process, or they may be removed from the sample, photographed with a digital camera, and measurements calculated from the images. Re-measurement of organisms by a second analyst is facilitated by using the digital images. The first 20 encounters per species per sample are measured as follows:

Cladocera: Length from the top of the head to the base of the caudal spine or to the end of the carapace.

Copepoda: Length from tip of the head to the insertion of spines into the caudal ramus.

Mysis: Carapace length, or the length from the tip of the head to the cleft in the telson.

Bythotrephes: Body length, excluding the caudal process.

Cercopagis: Body length, from the top of the eye to the end of the caudal claws.

NOTE: *If the organisms are curved or bent, several straight line measurements should be made and summed to obtain total length.*

8.1.5 Since the length/weight relationship for *Holopedium gibberum* was developed based on the length of the foot, body lengths are first multiplied by 0.25 before calculating weight.

8.1.6 *Copepoda nauplii* are assumed to have a constant weight of 0.400 µg (Hawkins and Evans, 1979).

8.2 Rotifer Biomass

8.2.1 Rotifer biomass (µg) is calculated according to A. Ruttner-Kolisko (appendix in Bottrell *et al*. 1976). For most rotifers, calculations use a formula with the general form:

$$Rotifer\ biomass\ (\mu g) = (length^3 \times FF) + (\%BV \times length^3 \times FF) \times 10^{-6} \times WW : DW$$

where:

µg	=	biomass of individual
length	=	total length in µm
FF	=	species specific formula factor (see Appendix 2)
% BV	=	volume of appendages as a percent of body biovolume (see Appendix 2)
10^{-6}	=	conversion to wet weight; assuming a density of 1
WW:DW	=	wet weight to dry weight conversion

8.2.2 A wet weight/dry weight conversion factor of 0.1 (Doohan, 1973) is used for all genera except *Asplanchna*, for which a factor of 0.039 (Dumont *et al*., 1975) is used.

8.2.3 For the genus *Collotheca*, width is measured, and the following formula used:

$$Collotheca\ biomass\ (\mu g) = (width^3 \times FF) \times 10^{-6} \times WW : DW$$

8.2.4 For the genera *Conochiloides* and *Conochilus,* both length and width are measured, and the following formula used:

$$biomass\ (\mu g) = (length \times width^2 \times FF) \times 10^{-6} \times WW : DW$$

8.2.5 For the genera *Filinia* and *Trichocerca*, both length and width are measured, and the following formula is used to take into account the biovolume of appendages:

$$Biomass\ (\mu g) = (length \times width^2 \times FF) + (\%BV \times length^3 \times FF) \times 10^{-6} \times WW : DW$$

8.2.6 At least 20 encounters per species per cruise per lake are measured, preferably across all stations. Measurements are made as follows:

8.2.6.1 Loricate forms: body length from corona to the opposite end at the base of spine (if present).

8.2.6.2 Non-loricate forms: body length from corona to the opposite end, excluding spines, paddles, "toes" or other extensions.

9.0 CALCULATIONS AND REPORTING

9.1 Zooplankton data are reported as number of organisms per cubic meter, which are calculated as follows:

9.1.1 Volume of water filtered

$$V = \alpha N_g A$$

where:

V = Volume of water filtered (m^3)
α = Flow meter calibration factor (see 9.2)
N_R = Number of revolutions (read from the flow meter dial)
A = Area of the mouth of the net (m^2) = 0.1963 m^2 for 0.5-m diameter net

9.1.2 Microcrustacean Densities

$$D = \frac{N \times S}{V}$$

where:

D = Density of organisms in numbers per cubic meter
N = Number of organisms
S = Split factor
V = Volume of water filtered (from 9.1.1)

9.2 Flowmeters are calibrated during each cruise (see Zooplankton Sample Collection SOP, LG402). The calibration factor is calculated by dividing tow depth by the average number of revolutions recorded during the tows. This information should be recorded in the field notebook for each cruise, and also entered into the shipboard data storage system.

9.2.1 The formula for flowmeter calibration is as follows:

$$\alpha = \frac{d}{N_{R\ (ave)}}$$

where:

α = Flowmeter calibration
d = Sample depth
$N_{R(ave)}$ = Number of revolutions, averaged for 20 calibration tows

9.3 Rotifer (and Nauplii) Densities

9.3.1 Calculate the densities of rotifers and nauplii using the following formula:

$$D = \frac{N \times V_s \times S}{N_A \times V}$$

where:

D = Density of organisms in number per cubic meter
N = Number of organisms
N_A = Number of 1-mL aliquots examined
V_S = Volume of sub-samples from which aliquots were removed
S = Split factor
V = Volume of water filtered (from 9.1.1)

9.4 Data Entry

9.4.1 All microcrustacean and rotifer calculations are made using a spreadsheet program such as Excel or a database program. The following items are to be submitted for data review:

9.4.1.1 A hard copy of all data entered as well as the calculated results

9.4.1.2 A floppy disk with all data

9.4.2 Backup/duplicate disks must be made of all data disks submitted to EPA.

10.0 QUALITY CONTROL AUDITS AND METHODS PRECISION

10.1 In general, ten percent of all samples analyzed are analyzed in duplicate by a second analyst. If a data set has less than 10 samples, at least one sample from that data set should also be analyzed in duplicate.

10.2 Samples are counted by the second analyst while still in the plankton wheel (or other counting chamber) or Sedgewick Rafter cell, so that only interanalyst variation is quantified, and not variation associated with sub-sampling.

10.3 Results from the second analyst are reported under the same sample number as the original sample, with the exception that the seventh character is replaced by a "Q".

10.4 Percent similarity will be calculated for the samples analyzed in duplicate by two analysts, according to the following formula:

$$PSC = 1 - 0.5\sum_{i=1}^{K} |a - b|$$

where:

a and *b* are, for a given species, the relative proportions of the total samples *A* and *B*, respectively, which that species represents.

10.5 It is expected that the two counts should have a similarity of 90%. If not, the reasons for the discrepancies between analysts should be discussed. If a major difference is found in how the two analysts have been identifying organisms, the last batch of samples that have been counted by the analyst under review may have to be recounted.

11.0 SAFETY AND WASTE DISPOSAL

11.1 Proper PPE should be worn in the laboratory while handling and preparing samples for analyses. Follow all laboratory waste disposal guidelines regarding the disposal of formalin (37% formaldehyde) solutions. **Do not discard formalin solutions into the sink unless previously diluted as directed by your laboratory health and safety officer**.

12.0 REFERENCES

12.1 Balcer, M.D., N.L. Korda and S.I. Dodson. 1984. Zooplankton of the Great Lakes. A guide to the identification and ecology of the common crustacean species, 174p. Univ. Wisc. Press. Madison.

12.2 Bottrell, H.H., A. Duncan, Z.M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson and T. Weglenska. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. 24: 419-456.

12.3 Brooks, J.L. 1959. Cladocera, p. 587-656. In: W.T. Edmondson (ed.) Freshwater Biology, 2nd Ed., Wiley, New York, pp. 1248.

12.4 Doohan, M. 1973. An energy budget for adult *Brachionus plicatilis* Muller (Rotatoria). Oecologia. 13: 35 l-362.

12.5 Dumont, H.T, van de Velde, I. and Dumont, S. (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton, and benthos of continental waters. *Oecologia* 19: 225-246.

12.6 Edmondson, W.T. 1959. Rotifers, p. 420-494. In: W.T. Edmondson (ed.) Fresh-water Biology, 2nd Ed., Wiley, New York, pp. 1248.

12.7 Evans, M. 1985. The morphology of *Daphnia pulicaria*, a species newly dominating the offshore southeastern Lake Michigan summer *Daphnia* community. Trans Amer. Micro. Soc. 104: 223-231.

12.8 Evans, M.S., D.W. Sell and D.I. Page. 1982. Zooplankton studies in 1977 and 1978 at the Donald C. Cook Nuclear Power Plant: Comparisons of preoperational (1971-1974) and operational (1975-1970) population characteristics. Univ. Michigan. Great Lakes Res. Div. Spec. Rep. 89.

12.9 Gannon, J.E. 1971. Two counting cells for the enumeration of Zooplankton micro-Crustacea. Trans Amer. Micros. Soc. 90: 486-490.

12.10 Hawkins, B.E. and Evans, M.S. (1979) Seasonal cycles of zooplankton biomass in Southeastern Lake Michigan. *J. Great Lakes Res*. 5 (3-4): 256-263.

12.11 Hudson, P.L., Reid, J.W., Lesko, L.T. & Selgeby, J.H. (1998) Cyclopoid and Harpacticoid Copepods of the Laurentian Great Lakes. *Ohio Biological Survey Bulletin NS* 12(2).

12.12 Makarewicz, J.C. & Jones, D.H. (1990) Occurrence of *Bythotrephes cederstromei* in Lake Ontario offshore waters. *J. Great Lakes Res*. 16(1): 143-147.

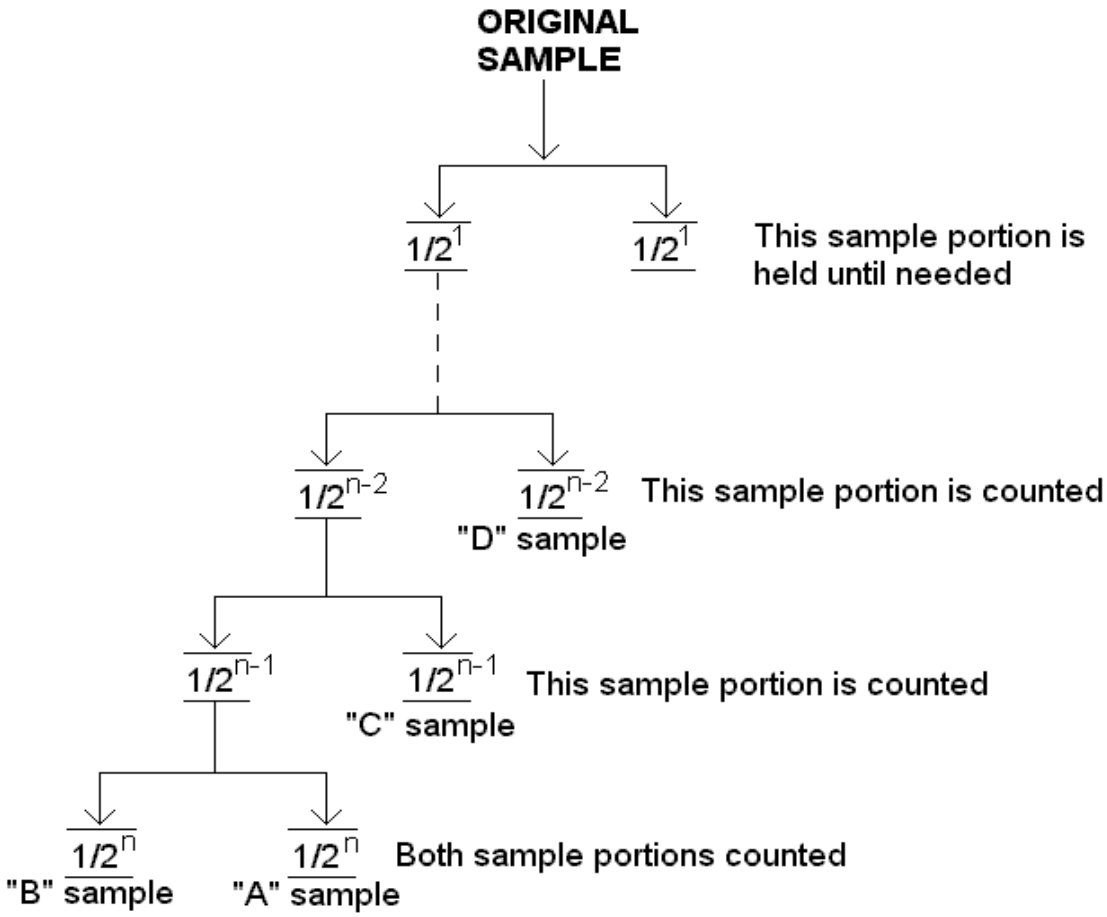
12.13 Ojaveer, H., L.A. Kuhns, R.P. Barbiero and M.L. Tuchman. 2001. Distribution and population characteristics of *Cercopagis pengoi* in Lake Ontario. J. Great Lakes Res. 27:10-18.

12.14 Rivier, I.K. 1998. The Predatory Cladocera (Onychopoda: Podonidae, Polyphemidae, Cercopagidae) and Leptodorida of the World. Backhuys Publishers, Leiden, The Netherlands, pp.213.

12.15 Shea, M.A. & Makarewicz, J.C. (1989) Production and trophic interactions of *Mysis relicta* in Lake Ontario. *J. Great Lakes Res*. 15(2): 223-232.

12.16 Stemberger, R.S. 1979. A guide to rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency, Rept. No. EPA 600/4-79-021, 185 pp.

Figure 1. ZOOPLANKTON SAMPLE SPLITTING DIAGRAM



LEGEND:

"n":	the final split level
"A" and "B" sample:	these sample portions are the two final sample volumes
"C":	the first preceding sample division
"D":	the second preceding sample division

NOTE: *The actual final sample division will be determined by the density of the organisms in the original sample. The first sample volume must have at least 200 organisms but not more than 400 organisms.*

Appendix 1. ZOOPLANKTON SAMPLE SPLITTING DIAGRAM

Species	LNA	B	Species used:	Reference
Alona guttata	4.543	3.636	*Chydorus sphaericus*	Rosen 1981
Alonella globulosa	4.543	3.636	*Chydorus sphaericus*	Rosen 1981
Bosmina longirostris	2.7116	2.5294	*Bosmina longirostris*	Bottrell *et al*., 1976
Bythotrophes cederstroemi	2.83	2.09	*Bythotrophes cederstroemi*	Makarewicz & Jones (1990)
Canthocamptus copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Canthocamptus robertcokeri	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Canthocamptus sp	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Cercopagis pengoi	-4.01738	3.01	*Cercopagis pengoi*	Simm & Ojaveer, unpubl MS
Ceriodaphnia lacustris	2.83	3.15	*Ceriodaphnia reticulata*	Pace & Orcutt 1981
Ceriodaphnia sp	2.83	3.15	*Ceriodaphnia reticulata*	Pace & Orcutt 1981
Chydorus sphaericus	4.543	3.636	*Chydorus sphaericus*	Rosen 1981
Cyclops bicuspidatus thomasi	1.4919	1.985	*Cyclops scutifer*	Persson & Ekbohm 1980
Cyclops copepodites	1.6602	3.968	*Mesocyclops edax*	Rosen 1981
Cyclops vernalis	2.2266	3.23	*Cyclops vernalis*	Rosen 1981
Daphnia galaeta mendotae	1.51	2.56	*Daphnia galaeta*	Dumont *et al*., 1975
Daphnia laevis	1.51	2.56	*Daphnia galaeta*	Dumont *et al*., 1975
Daphnia longiremis	1.0727	2.8915	*Daphnia longispina*	Bottrell *et al*., 1976
Daphnia pulicaria	1.9445	2.72	*Daphnia pulex*	O'Brien and deNoyelles 1974
Daphnia retrocurva	1.4322	3.129	*Daphnia retrocurva*	Rosen 1981
Daphnia sp	1.51	2.56	*Daphnia galaeta*	Dumont *et al*., 1975
Diaphanosoma sp	1.2894	3.039	*Diaphanosoma brachyrum*	Rosen 1981
Diaptomus ashlandi	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Diaptomus copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Diaptomus minutus	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Diaptomus oregonensis	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Diaptomus sicilis	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Diaptomus siciloides	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Epischura copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Epischura lacustris	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Ergasilus sp	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Eubosmina coregoni	2.7116	2.5294	*Bosmina longirostris*	Bottrell *et al*., 1976
Eucyclops agilis	1.4919	1.985	*Cyclops scutifer*	Persson and Ekbohm 1980

Species	**LNA**	**B**	**Species used:**	**Reference**
Eucyclops copepodites	1.4919	1.985	*Cyclops scutifer*	Persson and Ekbohm 1980
Eucyclops prionophorus	1.4919	1.985	*Cyclops scutifer*	Persson and Ekbohm 1980
Eucyclops speratus	1.4919	1.985	*Cyclops scutifer*	Persson and Ekbohm 1980
Eurycercus lamellatus	4.543	3.636	*Chydorus sphaericus*	Rosen 1981
Eurytemora affinis	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Eurytemora copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Halicyclops sp	1.4919	1.985	*Cyclops scutifer*	Persson and Ekbohm 1980
Harpacticoid copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Harpacticoida	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Holopedium gibberum	6.4957	3.19	*Holopedium gibberum*	Persson and Ekbohm 1980
Ilyocryptus sp	5.9913	7.942	*Ilyocryptus sordidus*	Rosen 1981
Leptodora kindti	-0.822	2.67	*Leptodora kindti*	Rosen 1981
Leydigia quadrangularis	4.543	3.636	*Chydorus sphaericus*	Rosen 1981
Leydigia sp	4.543	3.636	*Chydorus sphaericus*	Rosen 1981
Limnocalanus copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Limnocalanus macrurus	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Mesocyclops copepodites	1.6602	3.968	*Mesocyclops edax*	Rosen 1981
Mesocyclops edax	1.6602	3.968	*Mesocyclops edax*	Rosen 1981
Mysis relicta	-6.1709	2.86	*Mysis relicta*	Makarewicz and Shea (1989)
Polyphemus pediculus	2.7792	2.152	*Polyphemus pediculus*	Rosen 1981
Pontoporeia affinis	0	0		
Senecella calanoides	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Senecella copepodites	1.05	2.46	*Diatomus siciloides*	Pace & Orcutt 1981
Sida crystallina	2.0539	2.189	*Sida crystallina*	Rosen 1981
Tropocyclops copepodites	2.2266	3.23	*Cyclops vernalis*	Rosen 1981
Tropocyclops prasinus	2.2266	3.23	*Cyclops vernalis*	Rosen 1981
Tropocyclops prasinus mexicanus	2.2266	3.23	*Cyclops vernalis*	Rosen 1981

Appendix 2. ROTIFER BIOMASS FORMULA FACTORS

Species	FF	% BV
Ascomorpha ovalis	0.12	0
Asplanchna priodonta	0.23	0
Bdelloid Rotifera		
Brachionus	0.12	0.1
Brachionus angularis	0.12	0.1
Collotheca	1.8	0
Conochiloides	0.26	0
Conochilus unicornis	0.26	0
Copepod nauplii	0	0
Euchalanis spp	0.1	0.05
Filinia longiseta	0.13	0.01
Gastropus stylifer	0.2	0
Kellicottia longispina	0.03	0.015
Keratella cochlearis	0.02	0
Keratella crassa	0.02	0
Keratella earlinae	0.02	0
Keratella hiemalis	0.22	0.05
Keratella quadrata	0.22	0.05
Lecane sp.	0	0
Notholca foliacea	0.035	0
Notholca laurentiae	0.035	0
Notholca squamula	0.035	0
Ploesoma sp	0.1	0
Ploesoma truncatum	0.1	0
Polyarthra dolichoptera	0.23	0.1
Polyarthra major	0.23	0.1
Polyarthra remata	0.23	0.1
Polyarthra vulgaris	0.23	0.1
Pompholyx sulcata	0.15	0
Post-Veliger	0	0
Synchaeta spp.	0.1	0
Trichocerca cylindrica	0.52	0.006
Trichocerca multicrinis	0.52	0.006
Trichocerca similis	0.52	0.006
Trichocerca sp	0.52	0.006
Unknown Species #1	0	0
Veliger	0	0